

REVIEW

Chromosomal aberrations involving telomeres and interstitial telomeric sequences

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Telomeres are specialised nucleoproteic complexes localised at the physical ends of linear eukaryotic chromosomes that maintain their stability and integrity. In vertebrate chromosomes, the DNA component of telomeres is constituted by (TTAGGG)*n* repeats, which can be localised at the terminal regions of chromosomes (true telomeres) or at intra-chromosomal sites (interstitial telomeric sequences or ITSs, located at the centromeric region or between the centromere and the telomere). In the past two decades, the use of molecular cytogenetic techniques has led to a new spectrum of spontaneous and clastogen-induced chromosomal aberrations being identified, involving telomeres and ITSs. Some aberrations involve the chromosome ends and, indirectly, the telomeric repeats located at the terminal regions of chromosomes (true telomeres). A second type of aberrations directly involves the telomeric sequences located at the chromosome ends. Finally, there is a third class of aberrations that specifically involves the ITSs. The aims of this review are to provide a detailed description of these aberrations and to summarise the available data regarding their induction by physical and chemical mutagens.

Introduction

In the past two decades, the use of several molecular cytogenetic techniques has led to a new spectrum of spontaneous and clastogen-induced structural chromosomal aberrations being identified, involving telomeric sequences. This opened a new scenario in the analysis of the clastogenic effects of mutagens. The aims of this review are to provide a detailed description of these aberrations and to summarise the available data regarding their induction by physical and chemical mutagens. Although a previous review on the subject was published 5 years ago (1), the rapid development of this research area justifies a new, updated and more detailed revision. Before considering the chromosomal aberrations involving telomeric sequences, we will analyse some important issues concerning telomere biology that are necessary for understanding the main scope covered by the present review.

Telomeres and interstitial telomeric sequences

Telomeres are specialised DNA–protein complexes localised at the physical ends of linear eukaryotic chromosomes that maintain their stability and integrity (2). In vertebrates, they are composed of tandem arrays of duplex 5′-TTAGGG-3′ repeats (with the G-rich strand forming an overhang) bound by a specialised multiprotein complex known as ‘shelterin’ (3–7). The shelterin complex is charged with protecting chromosome ends from activating a DNA damage response, inhibiting inappropriate repair mechanisms and maintaining telomeric length and structure (5,6). Recent evidence shows that, besides telomeric repeats and shelterin, telomeres also comprise (UUAGGG)*n*-containing RNA molecules (*telomeric repeat containing RNA* or *TERRA*), a novel class of RNA for which several functions have been suggested (8–11). Many proteins involved in DNA repair are also associated to telomeres (5,7,8).

Telomeres serve multiple functions, including protecting the ends of chromosomes (12) and preventing chromosome fusion (13). The main function of telomeres is to distinguish natural chromosome ends from DNA double-strand breaks (DSBs) (i.e. they distinguish natural DNA ends from DNA ends resulting from breakage events) and thus promote genome stability. Therefore, cells respond to dysfunctional telomeres by undergoing senescence, cell death or genomic instability (7,14–21).

Telomere length is maintained by a dynamic process of telomere shortening and lengthening. Shortening can occur during semiconservative DNA replication in each round of cell division due to chromosome end processing by nucleases and incomplete DNA replication (i.e. the ‘end replication problem’, the inability of polymerases to fully synthesise the 5′ ends of DNA), whereas lengthening is primarily accomplished by the action of the ribonucleoprotein enzyme telomerase (22). Telomerase is a reverse transcriptase capable of compensating telomere attrition through *de novo* addition of TTAGGG repeats onto the chromosome ends by using an associated RNA component as template (*Terc*) (22). Telomerase is present in immortal cell lines, germline cells, stem cells, activated lymphocytes and most of the tumour cells analysed so far [see (23) for review]. Besides maintaining pre-existing telomeres, telomerase can catalyse the direct addition of telomeric sequences directly on to non-telomeric DNA, at or near the site of DSBs (24). This process is called ‘chromosome healing’, and has been observed in protozoans, yeast, plants, insects and mammals [see, e.g. (25,26)].

Telomere elongation can also occur in the absence of telomerase through the so-called ALT (for ‘alternative lengthening of telomeres’) mechanism, which involves homologous recombination between telomeres and has been described in several tumour cells and immortalised cell lines (27).

Decreased length of telomeric DNA sequences is sometimes associated with increased numerical chromosome aberrations (28). In addition, telomere shortening is accelerated by active oxygen species and ultraviolet radiation, which are thought to be major environmental causes of human telomere shortening

(29). It must be noted that telomere shortening is not the same as 'chromosome end loss' or true telomere loss since no terminal deletion events (i.e. no DSBs) are involved in this process. However, short telomeres act as if they were true DSBs and activate the DNA damage response, a characteristic of cells bearing DNA DSBs.

In some vertebrate species, the TTAGGG repeats are present not only in the terminal regions of chromosomes but also at interstitial sites of some chromosomes. These sequences are called interstitial telomeric sequences (ITSs), and includes those repeats located close to or at the centromeres and those found between the centromere and the telomere [see (30,31) for review]. The presence of ITSs has been assumed to be the result of tandem chromosome fusion (telomere–telomere fusions) during evolution (32–34) or the insertion of telomeric DNA within unstable sites during the repair of DSBs (35). ITSs are classified into different types according to their organisation: short, long subtelomeric, fusion ITSs and large blocks of these sequences (30,31). The latter, named pericentromeric ITSs or heterochromatic ITSs, comprises large blocks of telomeric sequences that are usually found within or at the margin of constitutive heterochromatin and has been found in species other than human [see, e.g. (32,36,37)]. The origin and formation of ITSs can be explained by different mechanisms, i.e. ancestral intrachromosomal rearrangements (inversions and fusions), differential crossing-over or repair of DSBs during evolution (30). It has been shown that ITSs do not represent a functional telomere (32) and, unlike terminal telomeric sequences, they seem not to be associated with the nuclear matrix (38). ITSs often colocalise with preferential sites of breakage, chromosomal recombination and DNA amplification (1,30,31).

Detection of telomeric sequences at the chromosome level

Telomeric repeats are usually detected at the chromosome level by using conventional fluorescence *in situ* hybridisation (FISH), peptide nucleic acid (PNA)-FISH or the primed *in situ* (PRINS) labelling reaction with a pan-telomeric probe (see below), i.e. a probe that recognises the regions of chromosomes possessing telomeric repeats [(TTAGGG)_n in vertebrate cells] and thus allows simultaneous visualisation of the telomeres of all chromosomes and the ITSs (if present) in a metaphase cell [see (1) and (39) for review]. However, it has been found that some immortalised cell lines, such as Chinese hamster ovary (CHO) cells, show no telomeric signals at the terminal regions of chromosomes, even when a technique as sensitive as PNA-FISH is applied to detect the telomeric repeats (40–45). The absence of signal at the terminal regions of CHO chromosomes has been ascribed to the fact that the copy number of telomeric sequences is too low to be detected effectively with currently available cytogenetic techniques (40,41,46). In effect, all immortalised Chinese hamster cell lines that have been analysed to date have extremely short telomeres (about 1 kb long) (41,43,47), whereas the average telomere length *in vivo* in the Chinese hamster is about 38 kb (47). It has been determined that the resolution of PNA-FISH methodologies on metaphase chromosomes is about 1000 bp of TTAGGG repeats (48) and that a functional telomere should be greater than 76 bp in length (49). Despite their very short telomeres, chromosomes from immortalised Chinese hamster cell lines do not show a high frequency of end-to-end chromosome fusions (43). This finding suggests that a few telomeric repeats are sufficient for telomere function.

Techniques for detecting telomeric sequences

As pointed out before, several techniques can be applied to chromosome preparations in order to detect the telomeric sequences present at the chromosomes. Since these techniques have been described elsewhere (1,39), they will be mentioned briefly here. Conventional FISH employing a synthetic oligonucleotide probe (TTAGGG or CCCTAA)_n has been successfully employed for *in situ* detection of telomeric sequences in the chromosomes of various vertebrate species [see, e.g. (32)]. However, the efficiency of telomere DNA probes has not been sufficient to extend conventional FISH beyond qualitative analysis of telomeric sequences or even to detect all human telomeres since less than 85% of telomeres could be identified in human lymphocytes (50,51).

The PRINS technique, based on the *in situ* annealing of synthetic oligonucleotides (CCCTAA)₇ to complementary nucleic acid sequences followed by primer extension in the presence of a hapten- or fluorochrome-labelled nucleotide (52,53), offers a faster method than conventional FISH for the *in situ* labelling of telomeric sequences and provides superior efficiency to conventional FISH for the detection of telomeric sequences and ITSs (54–56).

PNA-FISH for the detection of telomeric sequences is based on the use of a PNA telomere probe. PNA are synthetic DNA mimics in which the sugar phosphate backbone has been replaced by an uncharged and flexible polyamide backbone, and therefore, they are highly resistant to degradation by DNases, RNases, proteinases and peptidases (57). Because of the neutral backbone, PNA probes penetrate into the chromosome, and thus, a telomeric PNA probe provides a higher and much better efficiency in the detection of telomeric sequences than conventional FISH. The telomere PNA-FISH technique has been used extensively to detect telomeric repeats in human and other vertebrate cells, yielding a detection efficiency of 100% of human telomeres (58–61). In addition, PNA-FISH can be used to assess the length of telomeric repeats at individual chromosomes or the size of ITSs, i.e. quantitative telomere analysis using the quantitative (Q)-FISH technique (17,18,62,63). [For a discussion of the advantages and disadvantages of PNA-FISH over PRINS for telomere detection, see (1,39).]

In contrast, for the identification of some specific aberrations involving telomeric sequences, the chromosome orientation (CO)-FISH technique must be applied. CO-FISH is a strand-specific hybridisation technique commonly used to deduce the orientation of sequences along the chromosomes (64). In particular, the telomeric CO-FISH technique is used to discriminate between the different types of telomeric fusions and to detect telomere sister chromatid exchanges (T-SCEs) (13,65). In this procedure, cells are grown in the presence of 5-bromodeoxyuridine (BrdU) and/or bromodeoxycytidine (BrdC) for a single round of replication so that sister chromatids are unifarly substituted. After culture, chromosome preparations are exposed to UV light and exonuclease III (Exo III) treatment. Exposure of chromosomes to UV light in the presence of the photosensitising DNA dye Hoechst results in numerous strand breaks occurring preferentially at the sites of BrdU/BrdC incorporation, which serve as selective substrates for enzymatic digestion by Exo III. This results in the specific removal of the newly replicated strands, leaving the original (parental) strands largely intact. Thus, after CO-FISH, chromatids are single stranded and, when using a single-stranded

DNA or PNA probe, only one chromatid will show a hybridisation signal if the tandem repeats are oriented head to tail along the DNA strand (13,65). Accordingly, while standard FISH or PRINS with a telomeric probe produces four signals per chromosome, two at each end, CO-FISH typically yields just two signals, one at each end of the chromosome.

Telomere loss, breakage–fusion–bridge cycles and chromosomal instability

Telomere loss can generate chromosome instability through the so-called breakage–fusion–bridge (BFB) cycles, producing several types of chromosome rearrangements, including terminal deletions, inverted duplications, DNA amplification, duplicative and nonreciprocal translocations, and dicentric chromosomes, all of which have been associated with human cancer (66,67). In effect, when a chromosome loses a telomere due to a breakage event, the unprotected chromosomal end is exposed and, after replication, is thought to fuse with either another broken chromatid or its sister chromatid to produce a dicentric chromosome (Figure 1). The fused chromatids then form a bridge during anaphase that breaks as the two centromeres of the resulting dicentric chromosome are pulled to opposite poles. Since the break usually occurs in a place different to the site of fusion, one daughter cell receives a chromosome with a duplication on its end in the form of an inverted repeat, while the other daughter cell gets a chromosome with a terminal deletion. Because these broken chromosomes lack a telomere, following replication they will undergo sister chromatid fusion in the next cell cycle. Thus, BFB cycles can continue for multiple cell generations, leading to extensive chromosomal rearrangements, and terminate when the unstable chromosome eventually acquires a new telomere and so becomes stable (39,66,67). Lost telomeres after a BFB cycle can be acquired by several mechanisms, including nonreciprocal translocation, duplication/translocation, subtelomeric duplication or direct telomere addition (66,68).

Dysfunctional telomeres

Dysfunctional telomeres can be the source of genome instability and cancer (7). A dysfunctional telomere arises when it loses its end-capping function or becomes critically short, which causes chromosomal termini to behave like a DSB. A dysfunctional telomere elicits a DNA damage response by the activation of upstream kinases, DNA-PK, ATM (ataxia-telangiectasia mutated) and ATR (ataxia-telangiectasia and Rad3 related) (69,70). Dysfunctional telomeres are also potential substrates for homologous recombination (7). Alterations in the shelterin complex or other telomere-binding proteins (71), some DNA damage response proteins required for proper telomere protection (72), the structure of telomeric DNA (loss of telomeric sequences), the structure or activity of telomerase (73), TERRA (8–11) or the helicases (74,75) can give rise to dysfunctional telomeres. All these factors are involved in the production of telomere-related chromosomal aberrations (Table I). Telomere dysfunction at the chromosomal level is commonly assessed using metaphase chromosomes. However, not all telomere-involving chromosomal aberrations imply telomere dysfunction, but only those ones directly involving terminal telomeric repeats (see below for details).

Different studies have shown that dysfunctional telomeres are at the basis of structural chromosome aberrations and

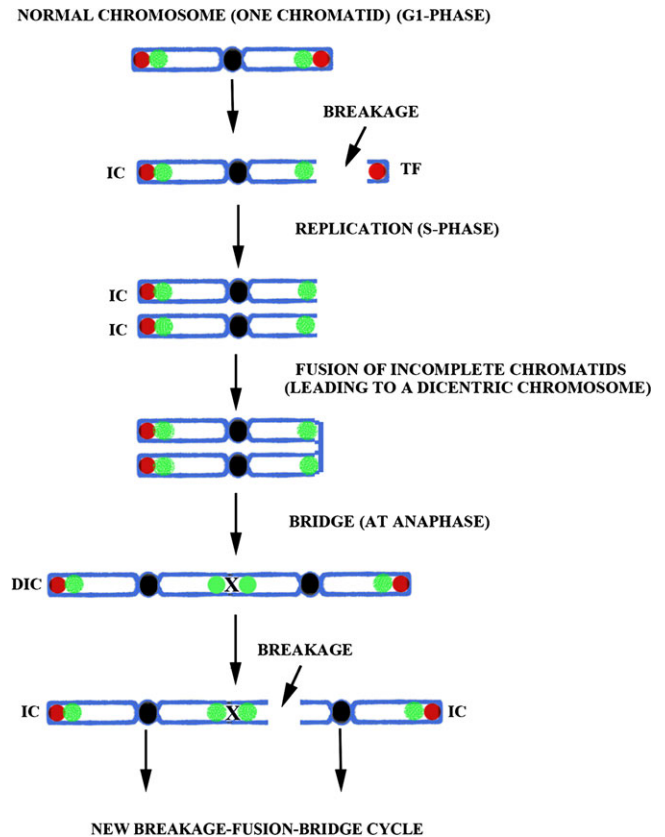


Fig. 1. Schematic representation of the breakage–fusion–bridge (BFB) cycle. Black circles represent the centromere of each chromosome; red circles, the telomeres (terminal telomeric repeat sequences); and green circles, the subtelomeric region of the chromosome. IC indicates incomplete chromosome (chromatid). Arrows indicate sites where the chromosome breaks have taken place. The 'X' indicates the site of fusion. The chromatid BFB cycle is initiated when a chromosome loses a telomere (i.e. a chromosome end) due to a chromosome break leading to a terminal fragment (TF). After the chromatid break occurs, the unprotected chromosomal end is exposed and, after replication, is thought to fuse with either another broken chromatid or its sister chromatid to produce a dicentric chromosome (DIC). Due to the presence of two centromeres, the fused chromatids form a bridge during anaphase that breaks when the two centromeres are pulled to opposite poles. Since the break usually occurs in a place different from that of the fusion, one daughter cell gets a chromosome with a duplication on its end in the form of an inverted repeat, while the other gets a chromosome with a terminal deletion, which can initiate another BFB cycle unless it acquires a new telomere. Following DNA replication in the next cell cycle, the sister chromatids fuse once again and thus the cycle continues, resulting in the amplification of DNA sequences located near the telomere and at interstitial sites, as well as the progressive accumulation of terminal deletions or TFs.

Table I. Factors that favour the formation of telomere-related chromosomal aberrations

Alterations in the structure of telomeric DNA (excessive telomere shortening or deletion and/or base damage in the telomere sequence)
Alterations in the shelterin complex
Loss of telomere-binding proteins
Alterations in the structure or activity of telomerase
Critical shortening of telomeres
TERRA (by inhibiting telomerase activity or by promoting telomere shortening)
Alterations in helicases
Alterations in the proteins linked to the cellular DNA damage response

chromosomal segment imbalances [see (21) for review]. Moreover, recent evidence indicates that telomere dysfunction plays a significant role in the genesis of numerical chromosome

aberrations since telomere shortening is linked to both chromosome lagging at anaphase and to nondisjunctional events between sister nuclei (21).
The presence of chromosome ends with undetectable TTAGGG hybridisation signals has been shown to be a good indicator of critically short and probably dysfunctional telomeres (76–79). It must be noted that telomere shortening does not always mean telomere dysfunction. Only when telomeric repeat loss gives rise to a defective telomere structure does a dysfunctional telomere appears. Chromosomes with dysfunctional telomeres tend to fuse with one another, producing dicentrics, which can give rise to the above-mentioned BFB cycles. Moreover, dysfunctional telomeres may result as a consequence of mutagen-induced telomere DNA damage (80–83). When telomeres become dysfunctional, cells can respond in three different ways: they can become senescent, enter crisis or begin BFB cycles that result in genomic instability (84–86).

Chromosomal aberrations involving telomeres and ITSs

Damaged telomeres and proper repair failure might result in telomere dysfunction, and when telomere protection fails, the consequences at the cellular level are readily visualised as chromosomal aberrations in mitotic cells. Recent evidence shows that telomere dysfunction can promote not only structural but also numerical aberrations (1,21,39). In this review, we will focus on the structural aberrations involving telomeres and ITSs. There are several types of structural chromosomal aberrations that can be identified using molecular cytogenetic techniques with a pantelomeric probe, i.e. a PNA or DNA probe that recognises the regions of chromosomes possessing telomeric repeats and thus allows simultaneous visualisation of the telomeres of all chromosomes and the ITSs (if present) in a metaphase cell (11) (Table II). Some aberrations involve the chromosome ends

and, indirectly, the telomeric repeats located at the terminal regions of chromosomes (i.e. true telomeres) (Figure 2 and Figure 3). A second type of aberrations directly involves the telomeric sequences located at the chromosome ends and results from telomere dysfunction (Figure 4 and Figure 5). Finally, there is a third class of aberration that specifically involves the ITSs (Figure 6) (1,39). Chromosomal aberrations involving telomeric sequences are usually identified using telomere FISH or PRINS, although chromosome painting or CO-FISH techniques must be used for a better discrimination of some types of aberrations (1,39).
When identifying and analysing structural chromosomal aberrations involving telomeric sequences, we have to bear in mind that the word ‘telomere’ is used to refer to the chromosome end itself and the telomeric repeat sequences located at the chromosome end (TTAGGG repeats in vertebrate chromosomes). Therefore, ‘telomere loss’ does not always mean true telomere or chromosome end loss, and sometimes this expression is used to refer to the extensive telomere shortening that occurs at the chromosome end. In both cases, chromosome end loss or extensive telomere shortening, no telomeric FISH or PRINS signals are seen in the chromosome end.

Chromosomal aberrations directly involving the chromosome ends and, as a result, terminal telomeric sequences: incomplete chromosome elements

These aberrations arise from the production of chromosome breaks at one or both chromosome ends of one or more chromosomes, and give rise to one or more acentric fragments (chromosome elements without centromere). These aberrations are collectively termed ‘incomplete chromosome elements’ or ICEs (Figure 2 and Figure 3) since they exhibit unrejoined or ‘open’ ends, in contrast to ‘complete aberrations’, whereby all broken chromosome ends find partners with which to rejoin. As a consequence, these aberrations are unstable chromosome-type aberrations. This group of aberrations represents the fraction of unrepaired or unresolved chromosome damage in a given cell population (exposed or not to a clastogenic agent) and includes incomplete chromosomes, terminal fragments and incomplete dicentrics (Figure 2 and Figure 3). Two main forms of ICEs can be distinguished, depending on the presence or absence of the centromere in the chromosome elements involved (1,39): centric ICEs, also termed ‘incomplete chromosomes’ or ICs (i.e. a chromosome lacking one or, less frequently, both ends) or acentric ICEs, also known as ‘terminal fragments’ or TFs (acentric fragments derived from a chromosome break at the terminal region of a chromosome and showing telomeric signals at one end) (see, e.g. Figure 2A). As previously mentioned, during the occurrence of BFB cycles, ICs and TFs are formed transiently (see Figure 1). The formation of ICEs is also involved in the process called ‘telomere capture’, a telomerase-independent and nonreciprocal translocation process that involves the addition of telomeres at the site of DSBs by subtelomeric cryptic translocations, undetectable by classical cytogenetic techniques (87,88). In telomere capture, broken chromosomes are stabilised by the transfer of telomeres from normal chromosomes [see (39) for details].

In complete metaphase cells, ICEs are always observed in pairs (1,39), which can be constituted by an IC plus a TF (Figure 2A), two ICs (accompanied by a compound fragment or CF, acentric fragment, which results from the fusion of two

Table II. Chromosomal aberrations involving telomeres and ITSs (see text for details)

A. Chromosomal aberrations directly involving the chromosome ends and, as a result, terminal telomeric sequences. These aberrations involve true telomere loss due to chromosome breaks occurring at the ends of chromosomes: ICEs: ICs (chromosomes lacking one or both ends), TFs and dicentric (or multicentric) chromosomes lacking one or both ends
B. Chromosomal aberrations directly involving terminal telomeric sequences and resulting from telomere dysfunction: Loss of a single telomere (no telomere FISH or PRINS signal; no breakage event involved) Loss of both telomeres of a chromosome end (no telomere FISH or PRINS signals; no breakage event involved) Chromatid telomere duplication Chromosome telomere duplication Telomere association Telomere fusion (chromosome and chromatid type): can be telomere–telomere or telomere–DSB fusions T-SCE (telomere recombination) Translocation of terminal telomeric sequences Amplification of terminal telomeric sequences (increase in the number or in the intensity of telomeric FISH or PRINS signals)
C. Chromosomal aberrations involving ITSs: Translocation or transposition of ITSs Amplification of ITSs Interstitial fragments (derived from breaks at the centromeric or pericentromeric region of a chromosome containing ITSs) Deletion or loss of ITSs

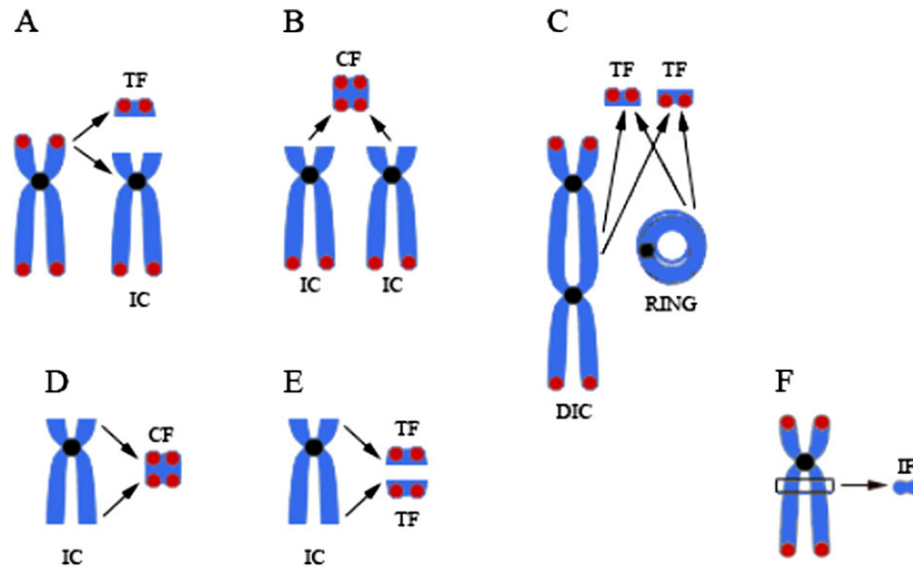


Fig. 2. Schematic representation of chromosomal aberrations involving the chromosome ends and, as a result, terminal telomeric sequences, as seen in metaphase cells using telomere FISH or PRINS (I): ICs and acentric fragments (see text for explanation). Black circles represent the centromere of each chromosome and red circles represent the telomeric sequences. The chromosomes are painted blue as if they were stained with DAPI counterstain. Arrows indicate sites where the chromosome breaks have taken place. (A) An IC accompanied by a TF. (B) A pair of ICs accompanied by a CF, also known as ‘proximal incomplete dicentric’. (C) A pair of TFs accompanied by a dicentric (DIC) or a centric ring chromosome, also known as ‘distal incomplete dicentric or ring’. (D) IC lacking both ends, accompanied by a CF, also known as ‘proximal incomplete ring’. (E) IC lacking both ends, accompanied by two TFs, also known as ‘proximal and distal incomplete ring’. TFs can join to another chromosome, promoting further instability (see, e.g. Figure 4O and P); (F) IF, which is not considered an ICE *per se* but has no telomeres and therefore lacks telomeric signals.

TFs and characterised by the presence of telomeric signals at both ends) (Figure 2B) or two TFs (accompanied by a dicentric or ring chromosome) (Figure 2C). Figure 2B illustrates a form of incomplete reciprocal translocation and is termed ‘proximal incomplete dicentric chromosome’ since the incompleteness corresponds to those ICs that did not form the expected dicentric chromosome, whereas the distal part of the chromosomes involved in the aberration (the two TFs) is complete (in the form of a CF). In contrast, Figure 2C depicts a form of ICs termed ‘distal incomplete dicentric or ring chromosome’ since in this type of aberration the incompleteness is restricted to the distal part of the dicentric or the chromosome forming the ring (i.e. the two TFs, which remain unjoined). As is the case for all dicentric chromosomes, distal incomplete dicentrics can be formed by the fusion of two chromosomes by their *p* or *q* arms. The dicentric depicted in Figure 2C is the result of the fusion of the *q* arm of one chromosome and the *p* arm of another chromosome. In some cases, the IC lacks both chromosome ends (89–91) (visualised as ICs without telomeric signals and symbolised as IC–/–, where ‘–’ represents a missed chromosome end or telomere) accompanied by one CF (‘proximal incomplete ring’, Figure 2D) or two TFs (‘proximal and distal incomplete ring’, Figure 2E). ICs also include those dicentric chromosomes that lack one or both ends (symbolised as dic+/- or dic-/-, respectively) (Figure 3A–P). For scoring purposes, an IC lacking both ends (Figure 2D and E) is counted as two ICs, whereas a dicentric chromosome lacking one end (Figure 3A–C, H, K and L) is counted as one IC (89–91). Of course, even though only dicentric chromosomes are illustrated in Figure 3, multicentrics lacking one or both ends are also possible. Dicentrics lacking both ends can give rise to dicentric rings with telomeric signals—if the original dicentric arose from a telomere–telomere (Figure 3I and J) or telomere–DSB (Figure 3M–P) fusion—or without them—if the original

dicentric arose from a DSB–DSB fusion (see Figure 3D–G). It is important to note that whole chromosome painting probes must be used to determine the origin of the acentric fragments involved in the aberration. Otherwise, some types of dicentrics lacking one or both ends are indistinguishable from each other. Thus, for example, for scoring purposes, the aberration depicted in Figure 3A is equal to that showed in Figure 3C (both are dic+/- and produce one CF and one TF), even though their mechanisms of origin are different.

Those acentric fragments not accompanying the formation of dicentrics or rings are termed ‘in excess’ acentric fragments, which might arise either from a complete exchange (an intra-arm intrachange, leading to an interstitial fragment or deletion) or an incomplete exchange or breakage (terminal deletions, leading to TFs or CFs), and their scoring provides additional information regarding chromosomal incompleteness. Besides the above-mentioned TFs and CFs, a third class of acentric fragment can be distinguished using FISH or PRINS with a telomeric probe, i.e. those ones derived from breaks occurring at interstitial sites of the chromosomes (i.e. the region comprised between the centromere and the telomere) and thus termed ‘interstitial fragments’ (IFs) (also known as acentric rings or double minutes, depending on their size). The aberrations can be recognised easily because they exhibit no telomeric signals after FISH or PRINS with a pantelomeric probe (1,39) (Figure 2F). After the formation of an IF, if the broken chromosome is not rejoined, an IC plus a TF results (like in Figure 2A). In some cases, IFs are considered as a form of ICs since in theory, IFs have two open ends, which may or may not have joined up to form a ring structure. However, previous evidence (92) strongly suggests that the majority of IFs lose the possibility of forming exchanges by the formation of a ring structure (acentric rings).

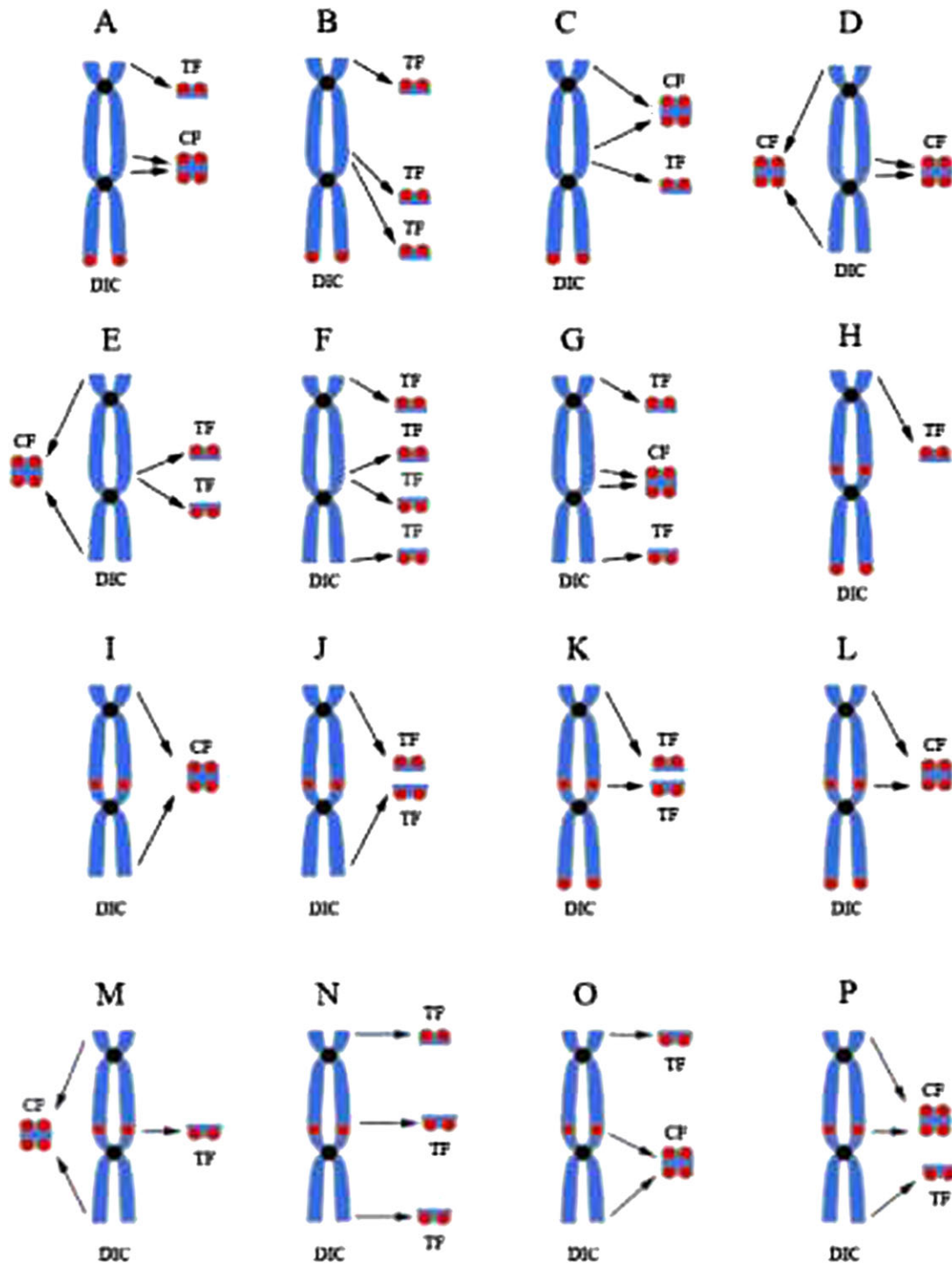
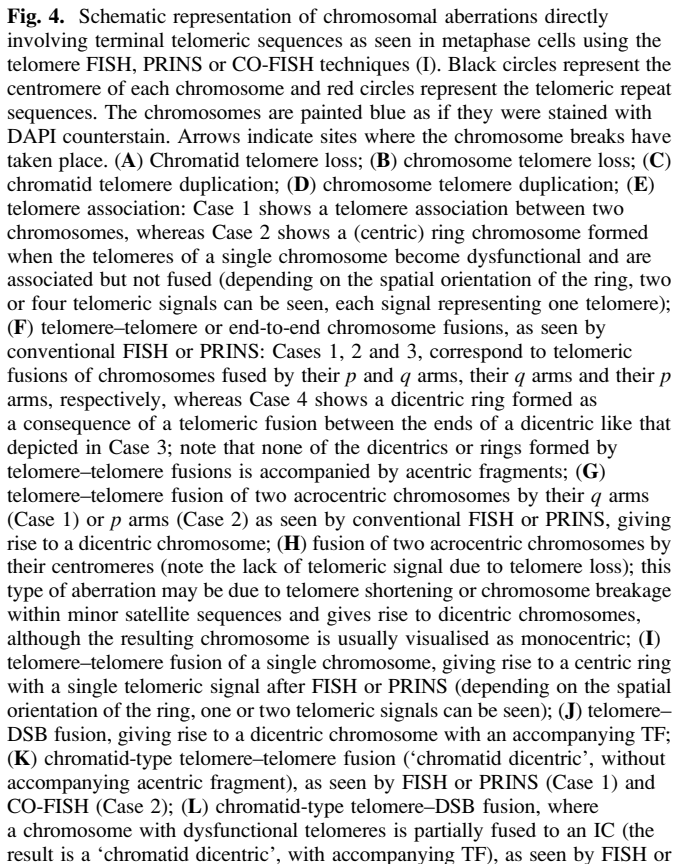


Fig. 3. Schematic representation of chromosomal aberrations involving the chromosome ends and, as a result, terminal telomeric sequences, as seen in metaphase cells using telomere FISH or PRINS (II): ICEs (continued): different types of dicentric chromosomes lacking one (dic+/-) or both (dic-/-) ends (see text for explanation). Black circles represent the centromere of each chromosome and red circles represent the telomeric repeat sequences. The chromosomes are painted blue as if they were stained with DAPI counterstain. Arrows indicate sites where the chromosome breaks have taken place. (A–G): Dicentrics lacking one (A–C) or two (D–G) ends, resulting from DSB–DSB fusions; (H–J): dicentrics lacking one (H) or two (I and J) ends, resulting from telomere–telomere fusions; (K–P): dicentrics lacking one (K and L) or two (M–P) ends, resulting from telomere–DSB fusions. In practice, to determine the origin of the acentric fragments formed, whole chromosome painting probes must be used.

Chromosomal aberrations directly involving terminal telomeric sequences

These aberrations result from telomere dysfunction, and as stated earlier, several factors may contribute to their production (Table I). Thus, the presence of one or more of these aberration types in a cell is a signature of dysfunctional telomeres (39,93).

This group of aberrations include loss of one or both telomeres (telomeric signals) of a chromosome end, duplication of telomeric signals at one or both ends of a chromosome or chromatid (extra telomere FISH or PRINS signals), association or fusion of telomeres of different chromosomes, telomere recombination at one or both ends of a chromosome, and



Loss of a single telomere (chromatid telomere loss) or both telomeres of a chromosome end (chromosome telomere loss). These aberrations result in the lack of telomere FISH or PRINS signals, although they do not involve the loss of the chromosome end since no breakage event has taken place in the aberrant chromosome. In chromatid telomere loss, one of the telomeres of a chromosome is missing and thus the corresponding chromatid shows no telomeric signal after FISH or PRINS with a telomeric probe (Figure 4A) [see, e.g. figure 1-I in (93) and figure 3-B-c in (95)]. In chromosome telomere loss, both telomeric signals of a chromosome end are missing [see Figure 4B—this review—and also figures 1-II in (93)]. These types of aberrations usually result from extensive telomere attrition or erosion, i.e. extensive telomere shortening at one or both chromatids in one of the chromosome ends. However, in some cases, these aberrations result from the translocation of terminal telomeric repeats of one chromosome to the telomere of another chromosome (i.e. nonreciprocal translocation or insertion of terminal telomeric sequences, like in the above-mentioned ‘telomere capture’ process), giving rise to a chromosome without a telomere (Figure 4A) and another one with chromatid telomere duplication (Figure 4C) or a telomere duplication (Figure 4D) and another chromosome without telomeres at one end (Figure 4B) (96). Since these types of aberrations do not necessarily involve a complete loss of telomeric sequences at the chromosome end, they should not be termed ‘telomere loss’, but ‘extensive telomere shortening’ or ‘lack of telomere signals’ instead. In fact, telomere signal-free ends after Q-FISH were correctly termed as ‘critically short telomeres’ and not ‘telomere loss’ (76).

PRINS (Case 1) and CO-FISH (Case 2); **(M)** telomere–telomere fusion between two chromosomes (Case 1) or within a single chromosome (forming a centric ring) (Case 2; depending on the spatial orientation of the ring, one or two telomeric signals can be seen), as seen by CO-FISH (compare M1 with F1, and M2 with I); **(N)** telomere–DSB fusion, giving rise to a dicentric chromosome with an accompanying TF, as seen by CO-FISH (compare with J); **(O)** chromosome-type telomere–DSB fusion between a chromosome and a TF (derived from an IC), as seen by CO-FISH; note that this can be seen as a telomere duplication at one end; **(P)** chromatid-type telomere–DSB fusion between a chromosome and a TF (derived from an IC), as seen by CO-FISH; **(Q)** T-SCE as seen by CO-FISH using one telomeric probe; **(R)** T-SCE as seen by CO-FISH using two telomeric probes, labelled with different fluorochromes (in this case, the site of T-SCE is labelled in a mixed colour). In Q and R, the arrow indicates the site of the T-SCE.

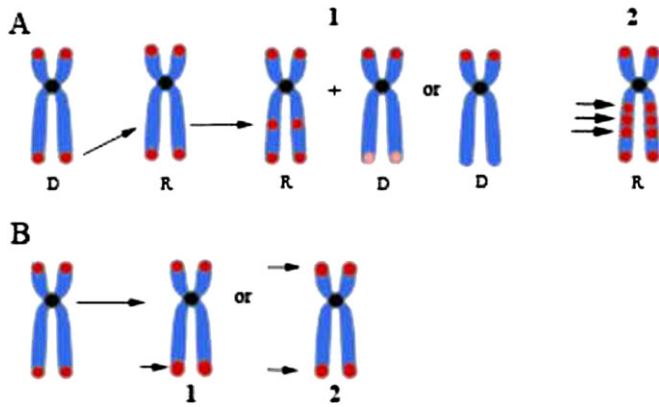


Fig. 5. Schematic representation of chromosomal aberrations directly involving terminal telomeric sequences as seen in metaphase cells using telomere FISH or PRINS (II): translocation and amplification of terminal repeats. Black circles represent the centromere of each chromosome and red circles represent the telomeric repeat sequences. The chromosomes are painted blue as if they were stained with DAPI counterstain. Arrows indicate sites where the translocation (in Figure 5A) or amplification (in Figure 5B) events have taken place. (A) Translocation involves only part of the telomeric repeats, which can give rise to a recipient (R) chromosome with one (Case 1) or several (Case 2) interstitial telomeric signals after FISH or PRINS (depending on the number of telomeres translocated) and a donor (D) chromosome with reduced telomeric signals (if the translocation involves only part of the telomeric repeats) or without telomeric signals at one end (if the translocation involves all of the telomeric repeats); (B) amplification of terminal telomeric sequences, occurring at one (Case 1) or both (Case 2) ends of a given chromosome.

Telomere association. This type of aberration implies that the telomeres (chromosome ends) of two different chromosomes are very close one to each other. Therefore, telomere associations are recognised in metaphase cells by the presence of two pairs of very close telomeric signals after hybridisation with a telomeric probe using FISH or PRINS, each pair of signals corresponding to a different chromosome (97,98) (Figure 4E, Case 1). Thus, to be scored as a telomere association, the telomeres of adjoining chromosomes have to be clearly distinguished from one another, i.e. four telomeric signals must be present, and the counterstain signal [in our case, DAPI (4',6-diamidino-2-phenylindole)] has to be discontinuous through the point of fusion (97,98) (Figure 4E, Case 1). Alternatively, if the telomeres of a single chromosome become dysfunctional—but not too short—they can be associated, but not fused, giving rise to a centric ring showing two telomeric signals—each one corresponding to one telomere—after conventional FISH or PRINS (Figure 4E, Case 2) [see, e.g. Figure 3 in (99) and Figure 1 in 100]. Telomere associations represent an important cytogenetic marker of human tumour cells (101). Also, the occurrence of telomere–telomere associations has been suggested to play a role in nuclear organisation (102). Telomere associations usually result from telomere shortening, which indicates that a minimum telomere length is required for proper telomere function, although the presence of these aberrations at metaphase in mammalian cells does not always correlate with telomere shortening (43,103,104).

Telomere fusion. Telomere or end-to-end chromosome fusions are the most frequent aberration resulting from telomere dysfunction (97,98) and can be visualised using conventional FISH or PRINS with a (pan)telomeric probe as events where the telomeres of adjoining chromosomes have fused into a single signal, one per chromatid (Figure 4F, Cases 1–3, and 6G) and the DAPI signal has to be continuous through the point of fusion (97,98). The chromosomes can fuse by their *p* or *q* arms, giving

rise to dicentric chromosomes of different shape and size (Figure 4F, Cases 1–3). Telomere fusions give rise to dicentric chromosomes without an accompanying acentric (compound) fragment (Figure 4F, G and I). Eventually, if the telomeres of this dicentric chromosome become dysfunctional, a dicentric ring can be formed (Figure 4F, Case 4). Telomere fusions are usually found in repair- and/or telomerase-deficient cells (99,100,105–108). Telomere fusions imply that telomeres became dysfunctional, but not that the telomeric repeats at the chromosome ends are completely lost. In fact, telomere fusions do not involve the loss of chromosome ends, as is the case for conventional dicentrics or ring chromosomes, where fusions between DSBs are involved. That is why telomeric signals are still present in the chromosomes involved in the fusion after FISH or PRINS with a telomeric probe. Moreover, not all telomere fusions are the result of telomere shortening. In fact, most of the end-to-end chromosome fusions occurring without telomere loss exhibit FISH signals that are brighter than those of not-fused telomeres, indicating that none of the telomeres involved in the fusion have suffered attrition.

Telomere fusions can be of two types: telomere–telomere, where two telomeres join to each other, which results in a dicentric or ring chromosome without accompanying acentric fragment/s (a CF or two TFs), or telomere–DSB fusion, where a telomere joins to a DSB (97) (see Figure 4). Although conventional FISH or PRINS can detect both events, to correctly discriminate between the different types of telomere fusions the CO-FISH must be applied (97,98). Thus, the two types of chromosome-type telomeric fusions, telomere–telomere (Figure 4F) and telomere–DSB (Figure 4J), can be clearly distinguished by simply looking at the CO-FISH hybridisation pattern (97). In the case of a telomere–telomere fusion, two blocks of telomeric DNA join in opposite orientations as a result of maintaining polarity (Figure 4M), so telomeric signals are detected on both chromatids of the dicentric chromosome at the point of fusion (Figure 4M, Case 1). In contrast, a telomere–DSB fusion produces a signal on just one chromatid (Figure 4N and O). In conclusion, under CO-FISH, a chromosome-type telomere–telomere fusion produces two interstitial signals, whereas a telomere–DSB fusion gives only one interstitial signal at the fusion point. Chromatid-type telomeric fusions (telomere–telomere, Figure 4K, Case 2, and telomere–DSB, Figure 4L, Case 2, and 4P) can also be identified by CO-FISH (97). However, unless one can be sure that the metaphase cell being scored is complete (no acentric fragment is lost), not even CO-FISH will be useful to discriminate between the different types of chromatid-type telomere fusions (compare Figure 4K, Case 2, and L, Case 2 but without the resulting TFs). In contrast, even if the acentric fragment is lost, chromosome-type telomere–telomere and telomere–DSB fusions are clearly distinguished by their CO-FISH pattern of hybridisation (see Figure 4M, Case 1, and L). As an illustrative example of the utility of the CO-FISH technique to discriminate between telomere–telomere and telomere–DSB fusions, imagine the aberration depicted in Figure 4J but without the TF (which has been lost). The only way to find which type of chromosome-type telomere fusion has taken place is to apply telomeric CO-FISH.

In addition, telomere fusions can be separated into two groups, chromatid- and chromosome-type fusions, depending on whether just one or both chromatids, respectively, are involved in the fusion event. In some cases, the telomeres of sister chromatids may become dysfunctional by persistent

cohesion and fusion (sister telomere fusion) (figure not shown). The telomere fusion event may occur between any type of chromosome, i.e. telocentric, acrocentric, metacentric or submetacentric, which can be fused by their *p* or *q* arms (see, e.g. Figure 4F, Cases 1–3). In contrast, the telomeres of a single chromosome can become completely dysfunctional and thus a centric ring with a single telomeric signal at the fusion point results (Figure 4I). It was shown that end-to-end fusions may form unstable rings but with a much lower frequency compared with dicentrics and that the average ratio of rings to dicentrics in whole genome was less than 1% (109). [For a more detailed description of the different types of telomere fusions, see (39).]

Telomere sister-chromatid exchange. T-SCEs are recombination events at telomeres, which imply an exchange of telomeric DNA between sister chromatids. This is a phenomenon first described in 1992 by Drets in CHO cells and human lymphocytes (110). At the molecular level, T-SCEs may be equal or unequal. In the first case, sister telomeres exchange equal quantities of DNA, whereas in the second case, one sister telomere becomes longer at the expense of the other. From a cytogenetic point of view, T-SCEs are easily identifiable using the CO-FISH technique (65,97) (Figure 4Q and R). Telomeric CO-FISH allows a single-stranded telomeric probe to hybridise to complementary telomeric DNA on one chromatid of each chromosome arm, producing a two-signal pattern instead of the four signals seen with conventional FISH. If a T-SCE occurred, a three-signal CO-FISH hybridisation pattern is expected since the effect of an SCE within telomere DNA is to split the hybridisation signal (Figure 4Q). As Figure 4R illustrates, when a true T-SCE has taken place, the sequential use of a C-rich telomere probe and a G-rich telomere probe produces a reciprocal pattern of hybridisation (65). The ‘spontaneous’ occurrence of T-SCE has been documented in different human and murine repair- or telomerase-deficient cell lines (111–115), and T-SCEs have been implicated as a marker of human tumours whose cells are sometimes forced to utilise the ‘ALT’ pathway to maintain telomere length (112,116). Moreover, it has been reported recently that hyper telomere recombination can dramatically accelerate replicative senescence of telomerase-negative cells and that this mechanism likely functions as a potent tumour suppressor (117).

Translocation of terminal telomeric sequences. A nonreciprocal translocation event or insertion of terminal telomeric sequences can occur when sequences derived from one chromosome insert into the interstitial region of another one (Figure 5A, Case 1). Depending on the number of telomeric sequences translocated, the donor (D) chromosome lacks telomeric signals or exhibits pale telomeric signals at one end, whereas the recipient (R) chromosome shows interstitial telomeric signals, which represent the translocated sequences (Figure 5A, Case 1). Alternatively, the terminal telomeric repeats of several chromosomes can be translocated into a single chromosome, which therefore shows several interstitial telomeric signals after telomere FISH or PRINS (see Figure 5B, Case 2). Thus, translocated terminal telomeric repeats are usually visualised as ITSs (42).

Amplification of terminal telomeric sequences. Sometimes, the terminal telomeric sequences become amplified, meaning that they increase in number in such a way that produces an increase in the size and/or the intensity of the hybridisation signals after

telomere FISH or PRINS compared with the normal telomeric hybridisation pattern of the cell type or species being studied (42) (Figure 5B). The amplification of terminal telomeric sequences can take place in one or both arms of a given chromosome (see Figure 5B, Cases 1 and 2, respectively). This type of aberration may arise through several mechanisms, including unequal SCE, BFB cycles or excision and reintegration events, i.e. the ‘rolling circle’ mechanism (1,39).

Aberrations involving ITSs

It has been shown that ITSs undergo frequent rearrangements including amplification, deletion and transposition or translocation [see (30) for review] (see Figure 6). In addition, many studies demonstrated that the ITSs may act as hotspots for breakage, recombination, rearrangement and amplification sites (46,118,119) and may participate in DNA repair and regulation of gene expression (40,120–122),

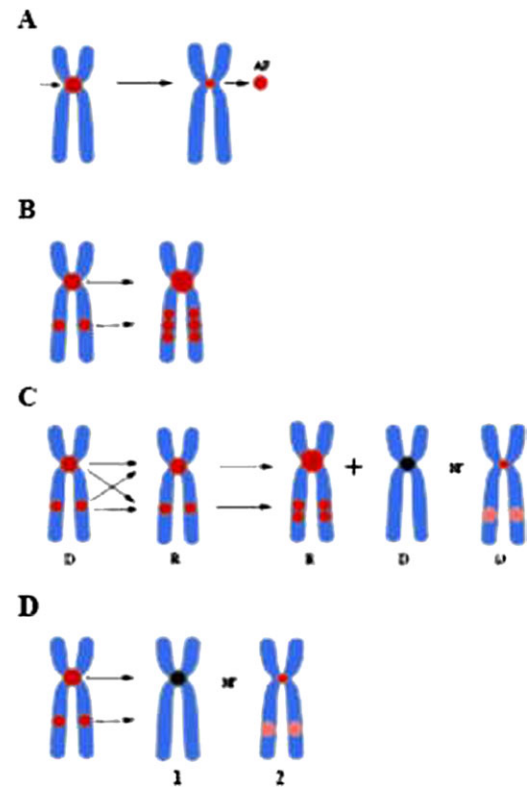


Fig. 6. Schematic representation of chromosomal aberrations directly involving ITSs as seen in metaphase cells using telomere FISH or PRINS. Black circles represent the centromere of each chromosome and red circles represent the telomeric repeat sequences. The chromosomes are painted blue as if they were stained with DAPI counterstain. Arrows indicate sites where the chromosome break (A), amplification (B) or translocation (C) events have taken place. (A) Interstitial fragment formed as a consequence of a chromosome break occurring at the centromeric or pericentromeric region of a chromosome containing ITSs. As a result, the acentric fragment appears entirely labelled with the telomeric probe; (B) amplification of ITSs, occurring at the centromeric or the interstitial region of a chromosome possessing ITSs (compare with Figure 5A, Case 2); (C) translocation or transposition of ITSs, which can give rise to a recipient (R) chromosome with one centromeric or several (in this case there are two) interstitial telomeric signals after FISH or PRINS, depending on the site of translocation, and a donor (D) chromosome with reduced telomeric signals (if the translocation involves only part of the telomeric repeats) or without telomeric signals at one end (if the translocation involves all of the telomeric repeats); (D) deletion of ITSs, which can take place at the centromeric or interstitial regions of chromosomes bearing ITSs, and can be total (Case 1) or partial (Case 2, where the chromosome exhibits reduced telomeric signals after FISH or PRINS).

although it has been shown that not all ITSs are hotspots for rearrangement or recombination (123–125). Azzalin *et al.* (35) proposed that short interstitial telomeric arrays—like those found in human cells—may not be fragile sites but simply mark sites of DSBs that occurred within unstable regions.

Interstitial fragments. Acentric fragments or minichromosomes containing telomeric repeats may arise if a breakage event occurs at the centromeric region of chromosomes having centromeric (heterochromatic) ITSs. Thus, the fragment derived from the centromeric region appears entirely and strongly labelled with the telomeric probe after FISH or PRINS (Figure 6A) (42,44,45). Centromeric breaks may also occur within the ITS heterochromatic block but without apparent chromosome break (visualised as a split signal after telomere FISH or PRINS) or at the centromeric region of a chromosome containing heterochromatic ITSs but not directly involving ITSs themselves (the telomeric signal remains as a single one) (figures not shown) (44,45).

Amplification and translocation of ITSs. As is the case with terminal telomeric sequences, spontaneous or induced amplification and translocation of ITSs may occur. Amplification of ITSs is visualised as an increase in the number and/or the size and intensity of the ITSs hybridisation signals after telomere FISH or PRINS compared with the normal telomeric hybridisation pattern of the cell type or species being studied (42,44–46,119,126,127) (Figure 6B), whereas translocation of ITSs means that a relocation of one or more pairs of ITSs signals compared with the normal telomeric hybridisation pattern of the species or cell type being studied has taken place (Figure 6C). In some cases, amplification of ITSs exhibits a similar pattern to that of the translocation of terminal telomeric sequences (compare Figures 5A, Case 2, and Figure 6B). Therefore, to distinguish a translocation from an amplification event in cells having both terminal and interstitial telomeric repeats, the number, distribution and intensity of telomeric signals after FISH or PRINS have to be recorded, and compared with the control pattern of these cells. As shown recently, the intensity of ITSs signals can be precisely determined using Q-FISH (44,45). Different mechanisms have been proposed to explain amplification of ITSs, including unequal SCE, BFB cycles, hyper-recombinogenicity of ITSs, or excision and reintegration events, i.e. the ‘rolling circle’ mechanism (1,30,39,44,45).

Deletion or loss of ITSs. Deletion of ITSs can occur at the centromeric or interstitial regions of chromosomes containing ITSs, and can be total (Figure 6D, Case 1) or partial (Figure 6D, Case 2). In the latter case, the chromosome exhibits reduced hybridisation signals after telomere FISH or PRINS. Deletion of ITSs can be identified by comparing the pattern of distribution of ITSs between untreated or control and clastogen-exposed cells.

Induction of chromosomal aberrations involving telomeres and ITSs by physical and chemical mutagens

In recent years, several studies were conducted using FISH with a telomeric probe in order to obtain information concerning the induction of chromosomal aberrations involving telomeres and ITSs by ionising radiation and chemical mutagens. These

studies have given new insights into the mechanisms underlying the formation of chemically and radiation-induced chromosomal aberrations [see, e.g. (30,31,44,45,90), and (1,39) for review]. Overall, these reports show that the involvement of telomeric sequences in the induced aberrations depends on the type of clastogen, the type of aberration and the type of sequence (terminal or interstitial) involved. A summary of the most important findings from the above-mentioned studies follows.

Chromosomal aberrations involving the chromosome ends or the telomeric sequences located at the chromosome ends

Several publications refer to the application of telomere FISH to analyse the induction of ICEs and IFs by low- and high-LET radiations and chemical mutagens (51,58–61,89,90,92,128–133) [see (1,39) for review]. These studies include the analysis of all types of asymmetrical chromosome aberrations induced by ionising radiation and the radiomimetic compound bleomycin in human lymphocytes and the ones induced by this compound and the antibiotics streptonigrin and streptozotocin in Chinese hamster cells.

It was found that the main form of ICEs (Figure 2) induced by clastogenic agents—irrespective of their mode of action—consists of pairs ICs + TFs (Figure 2A, Case 1) (59,128–131). The predominance of some form of ICE over the other ones might be explained by the attachment of the telomeres to the nuclear matrix [see (1) for discussion]. Moreover, all of the studies performed so far show that ICEs are the most frequent type of asymmetrical chromosome aberrations induced by chemical mutagens (128–131). The only exception was found in bleomycin-exposed human lymphocytes, where dicentric chromosomes predominated over ICEs (90). The above studies also demonstrated that IFs form a major class of low-LET, radiation-induced chromosomal aberrations, corresponding to about 80% of the excess acentric fragments induced by X-rays in human lymphocytes (58,59). In contrast, it was found that most of the excess acentric fragments induced by α -particles (high LET radiation) in human lymphocytes are of the terminal type (91). Moreover, about 50, 80 and 100% of excess acentric fragments induced by bleomycin, streptonigrin and streptozotocin, respectively, originate from incomplete exchanges or terminal deletions (128–131). On the contrary, an elevated induction of IFs or interstitial deletions (40% of the total deletions) was observed in human lymphocytes exposed to bleomycin (90). Thus, in contrast to that observed for high-LET radiation, the elevated induction of IFs seems to be a characteristic signature of the clastogenic effect of bleomycin and low-LET radiation, at least in human cells (58,59,90,128,131). Therefore, the pattern of induction of ICEs and excess acentric fragments depends of the clastogenic agent. Moreover, the ratio of ICEs/dicentrics in human lymphocytes was found to be different for bleomycin (0.27) (90), X-rays (0.38) (59), γ -rays (0.38) (89) and α -particles (1.00) (91). This shows that chromosomal incompleteness is higher in cells exposed to high-LET radiation than in cells exposed to low-LET radiation or bleomycin, suggesting that the damage induced by bleomycin or low-LET radiation is more likely to be repaired than that produced by high-LET radiation. However, a ratio of ICE/dicentrics of 27.6 has been reported for bleomycin-treated Chinese hamster embryo (CHE) cells (131). The different sensitivity to bleomycin between the CHE cells and human lymphocytes could be attributed to different factors (90), and suggests that the effect of clastogenic agents observed in cell lines cannot be directly extrapolated to human lymphocytes.

It has been recently reported in human lymphocytes exposed in G0 to γ -rays that cells bearing ICEs lacking a telomeric signal at one end are negatively selected during the G2/M checkpoint of the cell cycle (134), decreasing abruptly from G2 to M (seven times at 1 Gy and three times at 3 Gy of radiation). Therefore, although cells with ICEs can progress from G0 to G2, at the G2/M checkpoint they suffer a strong negative selection. This finding indicates that ICEs are the G0 ionising radiation-induced chromosome aberrations that are negatively selected in the G2/M checkpoint, making cells bearing such aberrations unable to reach mitosis. This has not been confirmed with other mutagens or cell types since no similar studies have been performed thus far.

In contrast, some studies have reported the presence of ICs lacking both telomeres and dicentrics lacking one or both telomeres in ionising radiation- and bleomycin-exposed cells (89–91) (see Figure 2 and Figure 3), although their frequency was found to be remarkably lower than that of conventional ICs (lacking only one end) and dicentrics (possessing both ends). Thus, despite their ‘open’ ends, chromosomes lacking both telomeres do not always form a centric ring and remain unrepaired.

Although ICs are unstable chromosome-type aberrations because they lack one or, less frequently, both chromosome ends, a few years ago Durante *et al.* (135) showed that chromosomes missing a telomere could be transmitted to the progeny of peripheral blood lymphocytes for at least three cell cycles after exposure and represented about 10% of all aberrations observed in the progeny of cells exposed to iron ions, and that terminal deletions and transmission of telomere-free chromosomes may be key events in determining late effects after exposure to high-charge and high-energy particles, which represent one of the main health risks for human space exploration. These findings suggest that ICs are not necessarily unstable aberrations, although further studies with clastogens other than ionising radiation should be performed to confirm this assumption.

Telomeric instability in the form of loss of telomere FISH signals, extra telomere FISH signals (chromatid telomere duplication = three signals at one telomere), extrachromosomal telomere FISH signals and end-to-end chromosome fusions have been observed in X-ray-exposed ATM-deficient mouse cells over many (>20) cell divisions post-irradiation (94). From these findings, the authors suggest that ATM deficiency makes telomeres vulnerable to breakage and proposed that telomeres are a critical target for induction of delayed chromosome instability by ionising radiation. Moreover, chromatid and chromosome telomere loss and duplications have been observed in immortalised normal human fibroblasts 14 days after X-ray exposure, suggesting that this type of radiation induces delayed telomere instability in human cells, i.e. telomeres can be destabilised several generations after X-irradiation (95) [see 93 for an excellent review on telomeres and radiosensitivity].

Concerning telomere associations, there are very few FISH studies reporting their induction by ionising radiation (136,137) and there are no data available regarding the induction of these events by chemical mutagens. This is very likely due to the fact that this type of aberration has been usually considered the same phenomenon as telomere fusion and, therefore, these two distinct events were scored as a single one. However, the most recent studies using molecular cytogenetic techniques make a clear and proper distinction between telomeric associations and telomeric fusions, although

the former are not always scored as chromosomal aberrations (98,106). The induction of telomeric fusions by X-rays has been demonstrated in different cell types using conventional FISH (94,138). However, no telomeric fusions were observed in normal human fibroblasts exposed to γ -radiation (50). The antitumour radiomimetic enediyne antibiotic C-1027 was also found to induce end-to-end chromosome fusions (139). Studies using CO-FISH to distinguish telomere–telomere and telomere–DSB fusions induced by chemical or physical mutagens are very scarce (97,98). These studies showed that γ -irradiation induces telomere–DSB fusions in cells derived from wild-type mice, mutant *scid* mice and a HTC75 human fibrosarcoma cell line expressing a TRF2 dominant-negative allele (i.e. with uncapped telomeres) (97), and that bleomycin induces chromosome-type telomere–DSB but not telomere–telomere (end-to-end) fusions in BRCA1-deficient human and mouse cell lines (98).

In particular, the study by Bailey *et al.* (97) demonstrated that functional telomeres protect chromosome ends from joining to radiation-induced DSB ends and that dysfunctional telomeres join to the ends of DSB induced by γ -irradiation, thus providing the first conclusive evidence of telomere–DSB fusions in mammalian cells.

At present, published data regarding the induction of T-SCEs by chemical or physical mutagens are scarce, and comes from results presented at the XV International Chromosome Conference (London, September 2004) by W. Wright (University of Texas) indicating that DNA damaging agents, such as ionising radiation, can induce T-SCEs [cited in (140)] and a recent paper by Berardinelli *et al.* (141) showing that high-LET radiation induces T-SCEs. Since ionising radiation normally does not induce SCEs, these findings suggest that, at telomeres homologous recombination mechanisms behave differently in comparison with the rest of the genome.

Chromosomal aberrations involving ITSS

Several external and internal factors, including type of clastogen and the nature of ITSS, decide the fate of ITSS either as unstable hotspots or stable sequences [see (30) for review]. For example, studies by Desmaze *et al.* showed that ITSS are not responsible for the chromosomal instability observed in human cells exposed to ionising radiation (124,125). As the following reports show, those ITSS usually involved in chromosomal aberrations are the ones located at the centromeric regions of chromosomes (heterochromatic ITSS).

Chinese hamster cells exposed to ionising radiation (46,142) and the radiomimetic clastogen bleomycin (42,44) showed that these agents induce the formation of IFs (Figure 2F) that appear strongly labelled with the telomeric probe after FISH. These findings suggest that centromeric regions containing ITSS are prone to breakage and recombination by ionising radiation and radiomimetic compounds [see (1) and (30) for review]. It has been reported that chromosome regions rich in heterochromatic ITSS are prone to breakage, fragility and recombination, both spontaneous and induced by ionising radiation (38,46,87,126,142–147), restriction endonucleases (40), mitomycin C and teniposide (VM-26) (145), and the radiomimetic compounds bleomycin and streptonigrin (42). In some cases, it could be demonstrated that the percentage of chromosomal aberrations involving ITSS was higher than expected based on the percentage of the genome composed by telomeric sequences (42,44,45,126,143). Moreover, telomeric FISH signals have been observed at the

site of breakage in chromatid exchanges like tri- and quadriradials (42,45,143). As suggested by Balajee *et al.* (40) and Fernández *et al.* (143), it is likely that the capacity of telomeric repeat sequences to form secondary structures within and between chromosomes (148) could account for their fragility and recombination. Some of the above-mentioned studies showed amplification of telomeric sequences at break-points and fragile sites (40,42,46). At least in the case of bleomycin and streptonigrin, this effect seems to be independent of telomerase activity (42). More detailed and recent studies in CHO cells exposed to bleomycin and streptonigrin showed that these antibiotics induce terminal as well as interstitial translocation and also amplification of telomeric sequences (44,45). The amplification of telomeric sequences by bleomycin and streptonigrin appears as strong telomeric signals after PNA-FISH and a significant increase in the size of ITSs (as measured by 'Q-FISH') in the chromosomes of exposed cells, and seems to occur mainly in the G1 or S phases of the cell cycle (44,45). Although several mechanisms have been proposed to explain amplification of ITSs [see (30,31) for review], the underlying mechanism involved in the amplification of telomeric repeats by bleomycin and streptonigrin in Chinese hamster cells remains to be established.

The above studies also showed that bleomycin and streptonigrin induce chromosome breaks at centromeric regions rich in heterochromatic ITSs, although these regions are not the preferential target of the clastogenic action of these compounds (44,45). Moreover, it has been observed that the involvement of heterochromatic ITSs in the aberrations induced by radiomimetic clastogens is not random since these sequences are preferentially involved in some types of aberrations. In effect, heterochromatic ITSs are preferentially involved in the chromosome- and chromatid-type breaks and chromatid exchanges induced by bleomycin and streptonigrin—taking into account the percentage of the genome covered by telomeric sequences—compared with other types of unstable aberrations induced by these compounds, and most of the chromosome breaks involving telomeric sequences in CHO cells induced by bleomycin occur at the centromeric region of chromosomes, whereas in streptonigrin-exposed cells these breaks occur outside the centromere (44,45).

Conclusions

The studies reviewed here show that the use of molecular cytogenetics allows for the identification of a new spectrum of aberrations that particularly involves the telomeric sequences of chromosomes. This opens a new scenario in the analysis of the clastogenic effects of mutagens. In effect, the identification and analysis of the aberrations described in the present review provide important new clues regarding the origin and mechanisms of formation of mutagen-induced chromosomal aberrations. For instance, through the use of telomeric FISH or PRINS (alone or in combination with pancentromeric and whole chromosome painting probes), it is now possible to precisely identify the origin and constitution of each acentric fragment induced, the presence of chromosomes without telomeres (lack of repair), the presence of telomere or telomere–DSB fusions, recombination events at the telomeres or alterations in the telomeric sequences (amplification, duplication, deletion, translocation). Undoubtedly, although current molecular cytogenetic techniques allow us to identify several types of aberrations involving telomeres, improvements in the resolution of these

techniques to a degree that allows us to detect very short telomeres will allow a more extensive and detailed analysis of these aberrations.

Overall, the studies reviewed here show that telomeres and ITSs play a significant role in the formation of chromosomal aberrations, and that the involvement of telomeric repeats in the induced chromosomal aberrations depends on the type of clastogen and the type of aberration considered. Therefore, the analysis of chromosome damage involving telomeres and ITSs should be seriously considered when the clastogenic effects of a given mutagen are being investigated.

Some unexplored or scarcely explored and interesting areas of research related with the topic outlined in the present review include the study of the relationship between chromosomal aberrations, telomeric sequences, telomerase activity and gene expression (i.e. genes linked to telomere maintenance) in mutagen-exposed cells; analysis of the long-term effects of mutagens on telomeres and ITSs; analysis of the induction of some types of chromosomal aberrations involving telomeres—like telomere–telomere and telomere–DSB fusions and T-SCEs—and elucidation of the mechanisms involved in the amplification and translocation of telomeric repeats induced by physical and chemical mutagens.

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